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No evidence for parental imprinting of mouse 22q11 gene orthologues

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Abstract

Non-mendelian factors may influence CNS phenotypes in patients with 22q11 deletion syndrome (22q11DS, also known as DiGeorge or Velocardiofacial Syndrome), and similar mechanisms may operate in mice carrying a deletion of one or more 22q11 gene orthologues. Accordingly, we examined the influence of parent of origin on expression of 25 murine 22q11 orthologues in the developing and mature CNS using SNP-based analysis in interspecific crosses, as well as quantification of mRNA in a murine model of 22q11DS. We found no evidence for absolute genomic imprinting or silencing. All 25 genes are biallelically expressed in the developing and adult brain. Furthermore, if more subtle forms of allelic biasing are present, they are very small in magnitude, and most likely beyond the resolution of currently available quantitative approaches. Given the high degree of similarity of human 22q11 and the orthologous region of mmChr16, genomic imprinting most likely cannot explain apparent parent-of-origin effects in 22q11DS.

Introduction

The mechanisms that underlie phenotypic variability in patients with 22q11 Deletion Syndrome (22q11DS; also known as DiGeorge or Velocardiofacial Syndrome) remain unknown. Parental origin of the 22q11 deletion may influence the severity of neuroanatomical and behavioral anomalies in 22q11DS patients (Eliez et al., 2001a; Eliez et al., 2001b; van Amelsvoort et al., 2001). In an MRI analysis of a limited sample of 22q11DS patients, maternal inheritance of 22q11 deletion is associated with quantitative changes in brain morphology, including a greater reduction in cortical grey matter volume (Eliez et al., 2001a), and also with increased severity of deficits in language skills (Glaser et al., 2002). This association with maternally inherited deletion suggests that one or more genes in the minimal critical deleted region for 22q11DS (Amati et al., 1999; Carlson et al., 1997; Matsuoka et al., 1998; Maynard et al., 2002b) may be preferentially expressed from the maternal chromosome. Such differences could result in dramatic reduction or complete loss of function when only the paternal chromosome remains. Accordingly, we asked if there are parent of origin effects on expression of individual 22q11 orthologues, especially in the developing or mature brain.

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Imprinting, the most extreme example of allelic bias, is often conserved between mouse and human genes (Morison et al., 2005; Wang et al., 2004; Yang et al., 1998). Thus, analysis of allelic expression of 22q11 orthologues in the mouse CNS-where comprehensive analysis in the developing and mature brain is feasible-should indicate whether imprinting is a significant feature of the multiple 22q11 genes that are expressed in the nervous system (Maynard et al., 2003). We used SNP analysis of interspecific crosses of two distinct mouse strains (*Mus musculus* and *Mus spretus*), as well as quantitative PCR analysis in mice carrying a deletion of the orthologous region of Chr.16 (Large Deletion-Lg.Del.; Merscher et al, 2001) to evaluate parent of origin effects on the full compliment of murine 22q11 orthologues. We found no evidence for imprinting or significant allelic biasing in any of 25 brain-expressed 22q11 orthologues.

Materials and Methods

Mouse breeding

Animals were handled in accordance with protocols approved by the UNC Institutional Animal Care and Use Committee. For SNP analysis, offspring of interspecific matings between *Mus musculus* (ICR, Charles River) and *Mus spretus* (Jackson Laboratory) were collected at embryonic day 16 (E16, night of mating = E0), postnatal day 0 (P0) or as adults at postnatal day 70 (P70). Individual whole brains were dissociated in Trizol (Invitrogen) for RNA extraction. Following extraction, RNA was DNAse treated (DNAfree, Ambion) to remove genomic DNA, and cDNA pools were prepared by reverse transcription (ImPromptII, Promega) using random hexamer primers (Invitrogen). For quantitative PCR analysis, brain samples from mice carrying a deletion of the syntenic region orthologous to 22q11 (LgDel; Merscher et al., 2001) on a C57-BL6 background crossed with wild-type C57-BL6 mice (Charles River) were harvested at P0 in Trizol. The sex of each sample was identified or confirmed by PCR for a Y-chromosome specific transcript (SMCY; 5′-CCAAGCCCAGTCCAATGTCCTCATC-3' and 5'-GGCAAGGTAGGGGGCTTCTTATGTC-3′).

SNP analysis, cDNA sequencing, and expression quantification

To identify single nucleotide polymorphisms (SNPs), PCR primers were designed to amplify segments of each CNS-expressed 22q11 orthologue (Maynard et al., 2003) in cDNA pools generated from adult brain RNA of ICR and *spretus* mice. PCR products from both ICR and *spretus* cDNA were agarose gel purified (QiaQuick Gel Extraction kit, Qiagen) and directly sequenced from either the forward or reverse primer on an Applied Biosystems ABI 3730 DNA analyzer (UNC Genomic Analysis Facility). Samples were processed in sets of 4 (1 male and female from *spretus* male X ICR female crosses; 1 male and female from ICR male × *spretus* female crosses), and 2 sets (n=8) were analyzed for each age. For all SNPs, allelic expression of each polymorphism was quantified by determining the relative maximum height of each chromatogram peak at the polymorphism. To account for differences between sequencing reactions, these values were normalized to the average height of the subsequent five peaks for the same nucleotide as the relevant SNP in the same sequence chromatogram (see Results and Fig. 2).

Statistical Analysis of SNP allelic expression

Normalized values reflecting the relative height of chromatogram peaks for multiple SNPs in the same gene were recorded as the allelic ratio for each expressed gene. Only these values were used for all statistical analysis of the SNP/interspecific cross/parent of origin data, and the same analytic method was used for each SNP. Each ratio was fit as the response in an analysis of variance (ANOVA) model using parent, time, sex, and all two- and three-way interactions. To determine whether parental effects were the same at all three ages, we also

examined simple effects of parent within age. If a transcript is imprinted or allelically silenced, the *Spretus*/ICR SNP ratios observed for *Spretus* male × ICR female offspring should differ significantly from ratios observed for ICR male × *Spretus* female offspring. Significance tests have not been adjusted for multiple comparisons, since p-values are used more as filters than as tests of hypotheses, and the analysis is hypothesis generating rather than hypothesis testing.

Quantitative real-time RT-PCR

To directly measure transcript levels in brain cDNAs from LgDel mice and littermate controls, we used quantitative real time RT-PCR analysis as described in detail in a recent examination of gene dosage in murine models of 22q11 deletion (Meechan et al., 2006, submitted). For the present analysis, the parental derivation of the deletion was recorded for each LgDel pup. RNA was collected from individual P0 mouse brains, and cDNA generated as described above. Expression was measured in these cDNA samples (maintaining their individual identity) with an ABI 7700 analyzer, using probes described previously (Meechan et al, 2005). The expression of each gene was calculated relative to a commonly used control gene (Gapdh). Comparisons were made between deleted and wild type mice using the Mann-Whitney test for unpaired samples.

Results

Identification of mouse single nucleotide polymorphisms

We analyzed 25 mouse 22q11 gene orthologues that are expressed in the developing and mature brain (Maynard, 2003), and identified SNPs between *Mus spretus* and *Mus musculus* (ICR) in transcribed sequences of 23 (Table 1). A total of 53 SNPs and 2 frame insertion/deletions were recorded based upon sequence analysis of approximately 17 kb of cDNA in the two strains (Table 2). Thus, we saw less than 3 SNPs per 1000 base pairs. This is a somewhat lower frequency than indicated in previous analyses of SNPs in multiple genomic sequences of mmChr.16, where a 1/50bp frequency was observed between *Mus spretus* and *Mus musculus* (Zhang et al., 2005). It is not clear whether this diminished SNP frequency reflects analysis of only transcribed sequences, or a higher than expected degree of homology for these transcripts. Sequence traces for multiple individual ICR and *spretus* mice were examined in detail to ensure that no intra-strain polymorphisms were evident. Not all SNPs were suitable for further allelic analysis. For our allelic expression analysis, we chose SNPs between 40-400 bp from the beginning of the sequence trace that produce clear individual peaks in the sequencing chromatogram, without interference ("bleed-through") from an adjacent peak. At least one polymorphism was identified in each gene except for *Tbx1*. *Tmvcf* has a 2-nucleotide insertion/deletion polymorphism instead of a single base change, but its expression can be analyzed by examining whether divergent chromatogram peaks following the insertion reflect either the maternal or paternal allele.

Analysis of imprinting/ allelic silencing

To investigate allelic silencing/imprinting in the 23 22q11 gene orthologues with one or more SNPs, we harvested whole RNA from the brains of *Spretus*/*ICR* intercrosses at three ages: embryonic day 16 (E16) during the process of cell proliferation, migration, and patterning; the day of birth (P0) during early maturation events, including process elaboration and synaptogenesis; and maturity (P70). To validate the identity of cDNA samples as well as sequencing accuracy, we analyzed an established allelically silenced gene, *Mkrn3*, from the Prader-Willi critical region, that is maternally silenced in both mice and humans (Jong et al., 1999). As expected, only the SNP from the paternal allele of *Mkrn3* was observed in sequencing chromatograms from ICR/*spretus* intercrosses (Fig 1, top left). In contrast, in the 23/25 transcripts that we could analyze using SNPs in interspecific crosses, both maternal and paternal alleles were present for each sequenced transcript (Figure 1). The expression of each

parental allele of the remaining two transcripts, *Ranbp1* and *Tbx1* could not be analyzed using SNPs and interspecific crosses (see below). Thus, for 23/25 mouse 22q11 orthologues, we did not see total or substantial silencing of either parental allele.

Measurement of Expression Bias in 22q11 orthologues

Most reports of parent-of-origin effects describe complete silencing of one allele; however, there has been at least one report of partial silencing (Yevtodiyenko et al., 2002). Thus, although we observed biallelic expression for 23/25 transcripts, we asked whether a more subtle form of allelic biasing—consistent departures from an approximately 1:1 expression ratio— can be correlated with parent of origin in our samples. We first determined whether we could improve upon semi-quantitative methods that rely upon measuring relative heights of peaks representing multiple SNPs in sequence chromatograms of reciprocal intraspecific crosses (e.g. Yevtodiyenko et al., 2002). To correct for variability between sequencing reactions, we measured the amplitude of each peak corresponding to specific nucleotides for the two parental SNPs in each transcript, and normalized these values to the amplitude of the adjacent (non-SNP containing) peaks for the same base (Figure 2A). To test whether such corrections accurately measure expression ratios, we produced an allelic "dilution series" using a fixed concentration of cDNA for one allele (ICR), and one-fold dilutions of the other (*spretus*) and analyzed SNP ratios in a representative 22q11 orthologue *Slc25a1* (CTP). The measured ratios of both *Slc25a1* SNPs accurately reflect the known two-fold dilutions of the starting material across the entire range of measured dilutions. Each of the multiple SNP measurements were within ±20% of each other, except at the most extreme (64:1) dilution, where the chromatogram trace for the more dilute ICR allele was barely detectable (Figure 2B). We also confirmed that a one-fold change in expression can be consistently detected with reasonably small measurement errors for 8 additional 22q11 genes by measuring the polymorphism ratios in 1:1 or 2:1 dilutions of *Spretus*:ICR cDNA (Figure 2C). For each of these eight, no individual measurement was greater than 0.5 fold from the known dilution, and the average of SNP measurements for three replicate samples was within 0.25 fold of the known relative concentration. Thus, it should be possible to reliably detect allelic biases of at least 1 fold using this analytic method.

Analysis of allelic expression ratio by SNP analysis

Allelic silencing for some transcripts has been shown to vary by age (Bennett-Baker et al., 2003; Weber et al., 2001). Accordingly, we analyzed expression ratios for each of the 23 transcripts in E16, P0, and P70 brains to assess whether there are allelic biases during brain development or in maturity (Figure 3). Some variation was observed in the measurements at each SNP (Figure 3); however, most values were approximately 1.0. The remaining differences in allelic ratios were less than onefold. The average maternal/paternal measurement ratio was never less than 0.8 (slight paternal bias) or greater than 1.25 (slight maternal bias). To determine whether these measurements indicate statistically significant biases, we performed an ANOVA analysis to determine whether *Spretus*/ICR ratios differ between mice from ICR Female × *Spretus* Male and *Spretus* female × ICR male crosses (Figure 4). We analyzed two females and two males for each reciprocal cross at each developmental age. Statistically significant ($P<0.05$) values were observed for SNPs in nine transcripts (*Arvcf, Cldn5, D16H22S680E, Dgcr2, Dgcr6, E2F6, Hira, Sept5*, and *Slc25a1*). However, if one discounts statistical significance where disparities exist between measurements of different SNPs in the same transcript (e.g. *D16H22S680E, Dgcr2, Hira, Slc25a1*), or where the absolute magnitude of the allelic ratio is within possible measurement error estimated by the dilution series shown in Figure 2B, (e.g. *Dgcr6, Sept5*), only three transcripts remain for consideration. Two, *Arvcf* and *E2f6* showed statistically significant biases at one age: *Arvcf* showed a slight paternal bias at P70 (Mat/Pat =0.89, P=0.04), and *E2f6* showed a slight maternal bias at E16 (Mat/Pat = 1.23, P=0.006). These transcripts, however, only have a single polymorphism for analysis, thus there

are no internal controls to further assess their validity. The third, *Cldn5*, showed modest maternal biases (maternal/paternal ratio = 1.06 - 1.18) across all ages, with similar statistical significance for each SNP ($p<0.02$). Thus, *Cldn5* may be a plausible candidate for subtle, partial allelic bias, particularly at E16.

Analysis of allelic expression ratio in LgDel +/- mice

Two 22q11 orthologues, *Tbx1, Ranbp1*, that have been implicated as candidates for 22q11 phenotypes (Lindsay et al., 2001; Maynard et al., 2002a; Merscher et al., 2001) were not suitable for an SNP-based analysis of allelic expression bias. There are no SNPs in the entire coding sequence of *Tbx1* for ICR versus *spretus* mice. *RanBP1* has three detectable SNPs; however, its expression is predominantly from the ICR allele in ICR/*spretus* crosses, regardless of parental origin (Figure 5A). Thus, we analyzed allelic expression using quantitative real-time RT-PCR (qPCR) in mice that inherited a heterozygous deletion orthologous to a 22q11 deletion (LgDel mouse; Merscher et al., 2001) from either their mother or father. In brain samples of P0 LgDel heterozygotes, expression levels of *Tbx1* and *Ranbp1* were approximately 50% of that in wild-type littermates, with no measurable difference between maternal and paternal deletions (Figure 5B). As a methodological confirmation, we demonstrated biallelic expression of *Hira* and *Prodh* with qPCR in Lg.del mice (Figure 5B). In all cases, there was no statistically significant difference between samples (Mann-Whitney U test; $Z>0.1$). Individual measurements of expression values for all four genes, measured by qPCR, vary by least ±10-20% among replicates, thus it is unlikely that qPCR could detect or confirm very small biases, such as the potential biases such as those suggested by the SNP analysis for *Cldn5*.

Discussion

There is a wide variation of phenotypes in 22q11 DS patients, even within multiple offspring that inherit an identical 22q11 deletion from a deleted parent (Digilio et al., 2003). Neuroanatomical (Eliez et al., 2001a) and behavioral (Glaser et al., 2002) analysis of individuals that inherited 22q11 deletions from either their mother or father suggest that at least some of variability in the brain phenotype may be caused by parental origin of the deletion. We assessed the most well understood cause of such a parental effect, genomic silencing/imprinting, in 25 murine 22q11 orthlogues and found no evidence that their expression is imprinted in the mouse brain. Furthermore, we have shown that if more subtle allelic biases exist for any of these transcripts, their magnitudes are small-there is apparently less than a one-fold difference in the expression level of the maternal and paternal transcripts. Thus, a simple hypothesis to explain phenotypic variability associated with parental derivation of the deletion in 22q11DS-imprinting of one or more genes in the deleted region-is not supported by our analysis.

We focused our study primarily on orthologues of the “minimal” 22q11 deletion region - an approximately 1.5 Mbp region thought to be deleted in virtually all 22q11DS patients (Shaikh et al., 2001). Two orthologues of genes in the “typically” deleted 3 Mbp region at 22a11 were included: *E2f6*, found in within a flanking low-copy repeat on 22q11; and *Crkl*, which has been suggested as a significant modifying gene (Guris et al., 2006; Moon et al., 2006); neither is imprinted in the developing, maturing, or adult mouse brain. Nevertheless, we did not analyze additional genes in the typically deleted region, or genes in other genomic loci that are infrequently associated with 22q11DS. Accordingly, it remains possible that there is an imprinted locus outside the minimal deletion region. To thoroughly evaluate this possibility, it would be essential to determine the full extent of the deletion, additional deletions or translocations, as well as the parent of origin, in further correlative studies of maternal versus paternal effects on 22q11DS phenotypes.

We found some statistical indication of modest allelic biases; however, they are small, inconsistent for different SNPs in the same transcript, and near the detection threshold for our methods-or any other commonly used measurement of allelic expression. Furthermore, they are not consistent at the three distinct ages evaluated here. The most likely candidate for significant and consistent expression bias is *Cldn5*, has a maternal/paternal expression ratio of 1.18 at E16 and approximately 1.06 thereafter. Although the allelic biases recorded for *Cldn5* showed the greatest statistical significance-particularly at E16-the absolute magnitude of this expression bias is quite small, and of uncertain biological significance. Although the functional contribution of Cldn5 to 22q11 DS phenotypes is unknown, its apparently transient, modest maternal bias is at least generally consistent with the apparent increase in severity of neuroanatomical (Eliez et al., 2001a) and behavioral (Glaser et al., 2002) phenotypes in patients who inherited the 22q11 deletion from their mothers.

It remains possible that one or more human transcripts from the 22q11 region are imprinted, even though their mouse orthologues are not. There is generally extensive conservation of imprinting between human loci and their murine orthologues. Nevertheless, several loci have been identified whose imprinting status differs between mouse and human (Morison et al., 2005). In most cases, however, changes in imprinting appear to be due to substantial alterations in genomic structure between the two species, such as the elimination or addition of an adjacent gene. All of the genes we have analyzed in mouse are present in the human genome. The segment of mouse chromosome 16 that is orthologous to human 22q11 is highly conserved (Lund et al., 2000; Puech et al., 1997; Roe et al., 2003; Sutherland et al., 1998), and there are relatively few genomic rearrangements or deletions that might alter transcription. Furthermore, our recent analysis (Meechan et al., 2006, submitted) indicates that putative regulatory regions of human 22q11 share significant homology with their orthologues on mouse chromosome 16, including binding motifs for specific transcription factors. Finally, the temporal and spatial expression profiles of subsets of 22q11 genes in human parallel those seen in mouse (Maynard et al, 2003; Meechan et al., 2005, submitted). Together, these observations diminish the possibility of significant divergence in imprinting of 22q11 genes and their murine orthologues.

We limited our analysis of 22q11 orthologue allelic expression to only one tissue-the brain-among many that are phenotypically compromised in 22q11DS. For some genes, imprinting results in uniform allelic silencing across tissues throughout life; nevertheless, allelic biases that defy these conventions have been reported for genes that are highly conserved in mouse and human (e.g. Albrecht et al., 1997; Deltour et al., 1995). Given the cytological and functional diversity of cell classes in the brain, however, transcripts in subsets of cells at distinct developmental stages could potentially have significant allelic bias or even complete allelic silencing. Such biases would be difficult to resolve in whole tissue samples due to biallelically-expressed transcripts of the gene of interest from adjacent cell classes. This sort of individual cellular difference can only be reliably confirmed by careful analysis of cellular expression in heterozygously deleted individuals (Albrecht et al., 1997), or by using single cell expression measurements (e.g. Chess et al., 1994). Thus in whole brains, complete imprinting of a transcript in a small population of cells-which might have a dramatic impact on relevant phenotypes in heterozygously deleted individuals-might be observed as a subtle, difficult to substantiate expression bias. Nevertheless, our SNP analysis in the normal offspring of intraspecific crosses as well as our qPCR analysis in mice carrying a deletion that models 22q11DS suggests that widespread imprinting of 22q11 genes is unlikely to influence phenotypic variability in 22q11DS.

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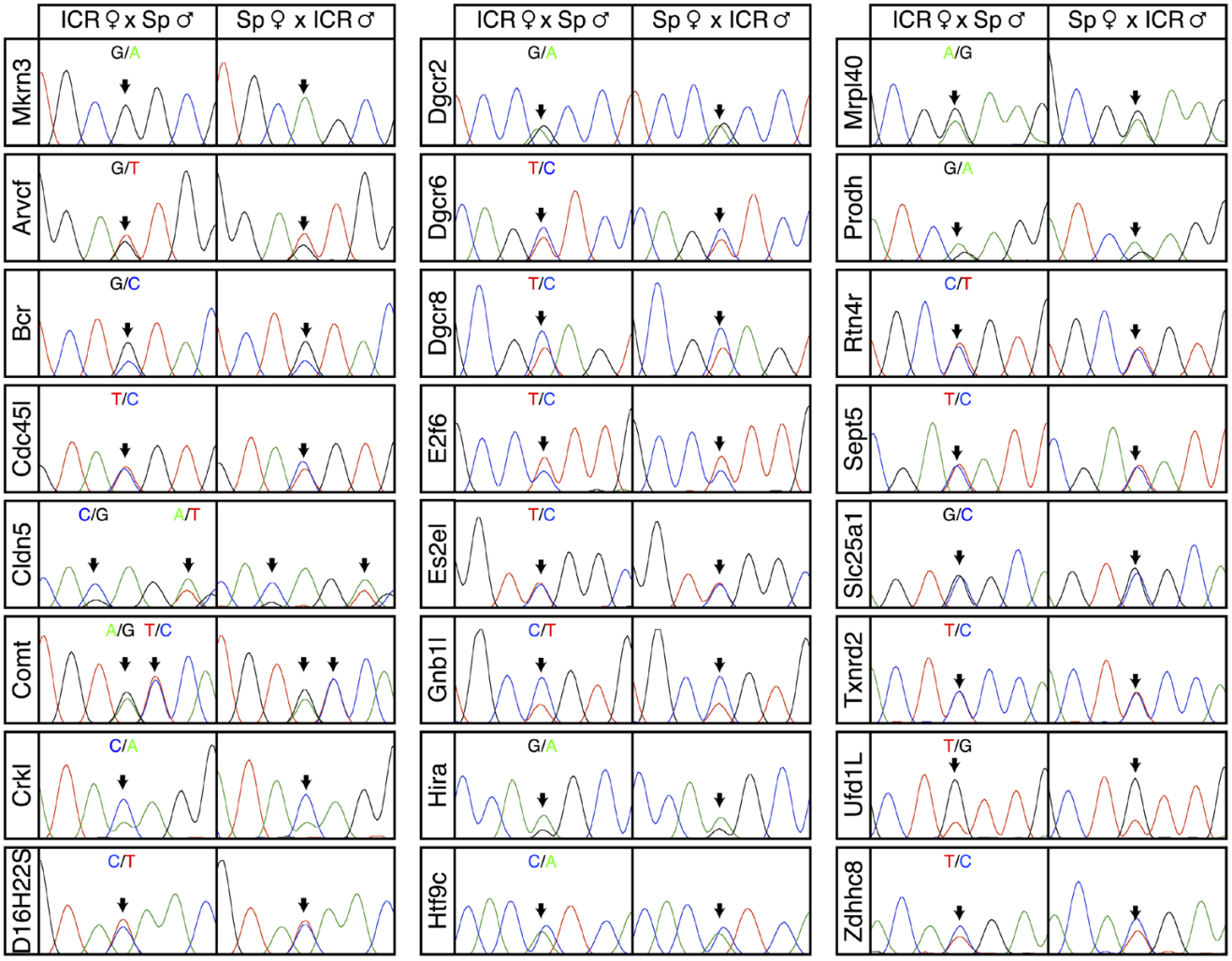

Figure 1.

Analysis of alleleic expression of 22q11 orthologues. Sample sequence chromatogram traces from P70 *Mus spretus* × *Mus musculus* (ICR) crosses. Two sample traces are presented for each transcript, the left hand trace from a cross with a ICR female, the right hand trace from a cross with an *Spretus* female. Polymorphic base pairs are noted by an arrow and the base change involved is denoted as (*Spretus* base/ICR base). At the top left is a known imprinted transcript (*Mkrn3*), where only the paternally inherited allele is detectable. For the other 23 transcripts shown, both maternal and paternal alleles are detected.

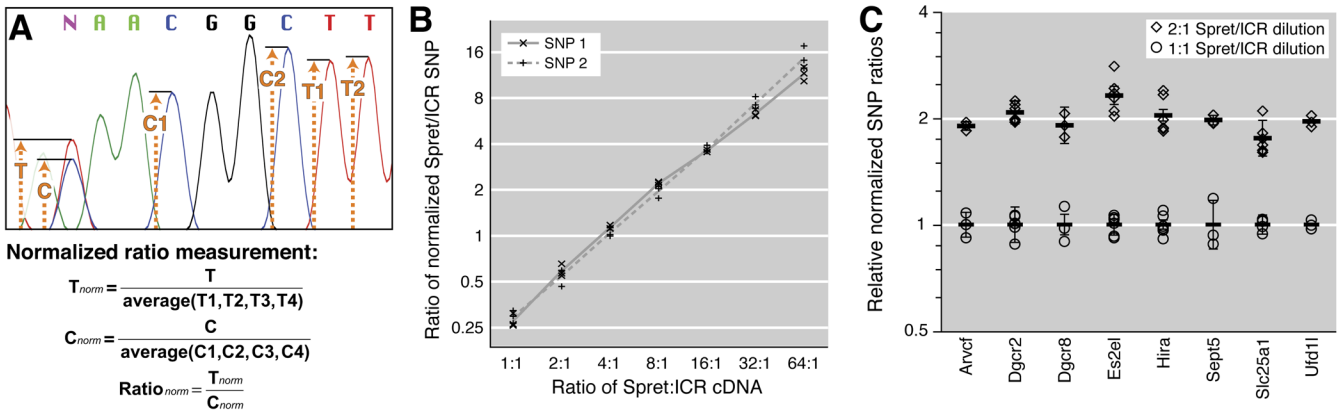

Figure 2.

Measurement of 22q11 orthologue polymorphisms using SNPs and sequence chromatograms. A. Measurement of expression ratio from sequencing chromatograms. The maximal amplitude of each expressed base at the site of a SNP is measured, and normalized to the average amplitude for the four subsequent peaks of that base to account for trace-to-trace variability. B. Measurement of expression ratio by sequence analysis from a dilution series of ICR and *spretus* cDNA. Measurements were made for the two polymorphisms in *Slc25a1* over a 64-fold change in cDNA ratios. Starting concentrations were not precisely quantified, but both cDNAs were produced using identical methods, and the Spretus cDNA was diluted 1:4 before preparing the dilution series. Measured values are displayed on a log scale for clarity. C. Expression ratios of 8 additional 22q11 orthologues were measured from two dilutions (1:1 and 1:2) of *spretus* and ICR cDNA. An approximate 50% reduction in relative expression was observed for all 8 transcripts.

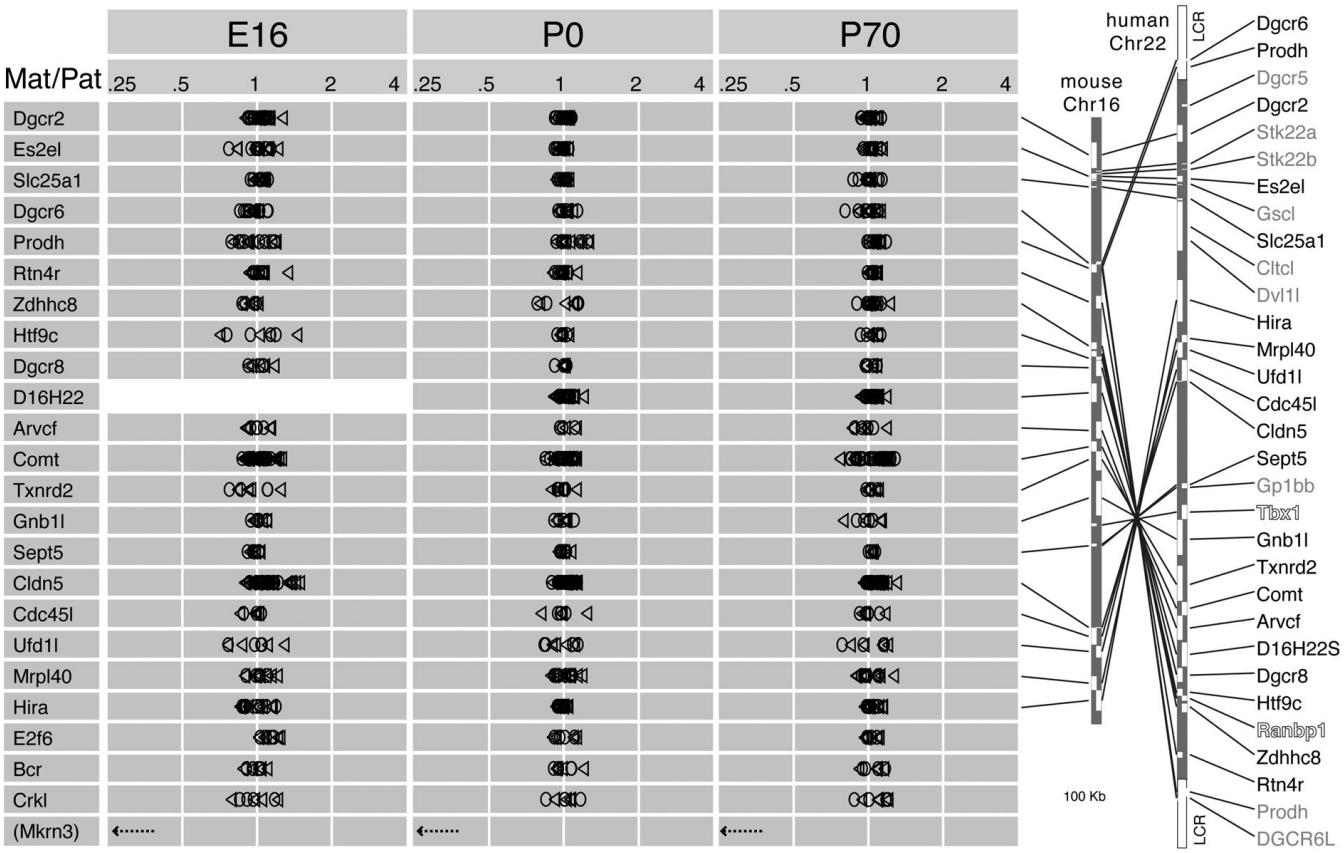

Figure 3.

Analysis of expression bias by SNP analysis for 23 22q11 orthologues in mice from ICR/*spretus* intercrosses in the brains from individuals at embryonic day (E) 16, postnatal day (P) 0, and in adulthood (P70). Ratios of normalized chromatogram measurements (see methods) are displayed on a log scale. Each point represents the deviation from average (equal allelic expression) for an individual sample. Points to the left of equal (Mat/Pat = 1) represent a paternal expression bias, as observed for *Mkrn3*. Expression of T10 at E16 is too low to permit reliable detection, and is thus excluded from analysis.

	E16		P0		P70		All Ages
	M/P	P=	M/P	P=	M/P	P=	P=
Arvcf	0.99	0.916	1.02	0.657	0.89	0.040	0.152
Bcr	0.95	0.471	1.01	0.869	1.04	0.220	0.302
Cdc45l	0.90	0.182	0.96	0.634	0.95	0.381	0.130
Cldn5	1.18	0.046 0.002 0.002 0.003 0.010	1.06	0.333 0.135 0.538 0.089 0.146	1.08	0.030 0.060 0.880 0.067 0.309	0.007 0.001 0.019 0.001 0.007
Comt	0.97	0.477 0.958 0.954 0.739 0.726	1.03	0.487 0.921 0.776 0.539 0.606	1.06	0.560 0.202 0.230 0.113 0.351	0.743 0.401 0.606 0.678 0.304
Crkl	0.95	0.504	1.02	0.953	1.08	0.958	0.746
D16H22S680E (T10)			1.05	0.495 0.235 0.137 0.049	1.02	0.314 0.902 0.287 0.119	0.805 0.348 0.726 0.021
Dgcr2	1.06	0.013 0.980 0.241	1.02	0.717 0.508 0.767	0.97	0.705 0.610 0.307	0.121 0.940 0.271
Dgcr6	0.93	0.141 0.178	1.03	0.642 0.101	0.94	0.041 0.048	0.896 0.167
Dgcr8	1.02	0.604	1.00	0.925	1.00	0.438	0.836
E2f6	1.23	0.006	1.01	0.903	1.00	0.124	0.306
Es2el	0.98	0.494 0.897	0.97	0.380 0.918	0.99	0.679 0.634	0.261 0.683
Gnb1l	1.02	0.885	0.99	0.943	0.91	0.868	0.957
Hira	0.93	0.665 0.007	0.97	0.686 0.643	1.04	0.337 0.082	0.563 0.311
Htf9c	1.00	0.992	0.98	0.856	1.01	0.533	0.645
Mrpl40	1.04	0.791 0.460	1.01	0.259 0.733	0.96	0.764 0.492	0.495 0.529
Prodh	0.95	0.460 0.561	1.10	0.089 0.353	1.05	0.974 0.950	0.553 0.864
Rtn4r	1.04	0.741 0.209	0.97	0.422 0.719	1.03	0.532 0.823	0.316 0.680
Sept5	0.93	0.019	1.00	0.988	1.02	0.519	0.075
Slc25a1	1.07	0.037 0.282	0.99	0.981 0.565	0.94	0.983 0.256	0.208 0.711
Txnrd2	0.91	0.312	0.97	0.640	1.01	0.759	0.306
Ufd1L	0.91	0.490	0.96	0.833	0.97	0.942	0.631
Zdhhc8	0.87	0.175	0.97	0.734	0.96	0.281	0.709

Figure 4.

Statistical correlation of expression bias with parent-of-origin at three distinct ages. Three columns are presented, one for each age (E16, P0, P70). On the left hand side of each column is a net estimate of expression bias (Maternal/Paternal) computed by dividing the average (Spretus/ICR) ratio for the *Spretus* female × ICR male crosses by the average (Spretus/ICR) ratio for the *Spretus* male × ICR female crosses. If expression is completely unbiased, this ratio would be expected to be 1; ratios smaller than 1 suggest increased expression from the maternal transcript. For statistical analysis, each polymorphism was analyzed separately. ANOVA analysis was used to determine the probability that the SNP ratios (corrected for measurement error) from *Spretus* female crosses were statistically different from ICR female crosses.

Significance across all ages (n=24 individuals) is listed at the right; while the probability for the subset of ratios at each age (n=8 individuals) is listed in each column. Statistically significant P values (P<0.05) are highlighted by dark grey shading; lighter shading indicates a potential trend (P<0.10).

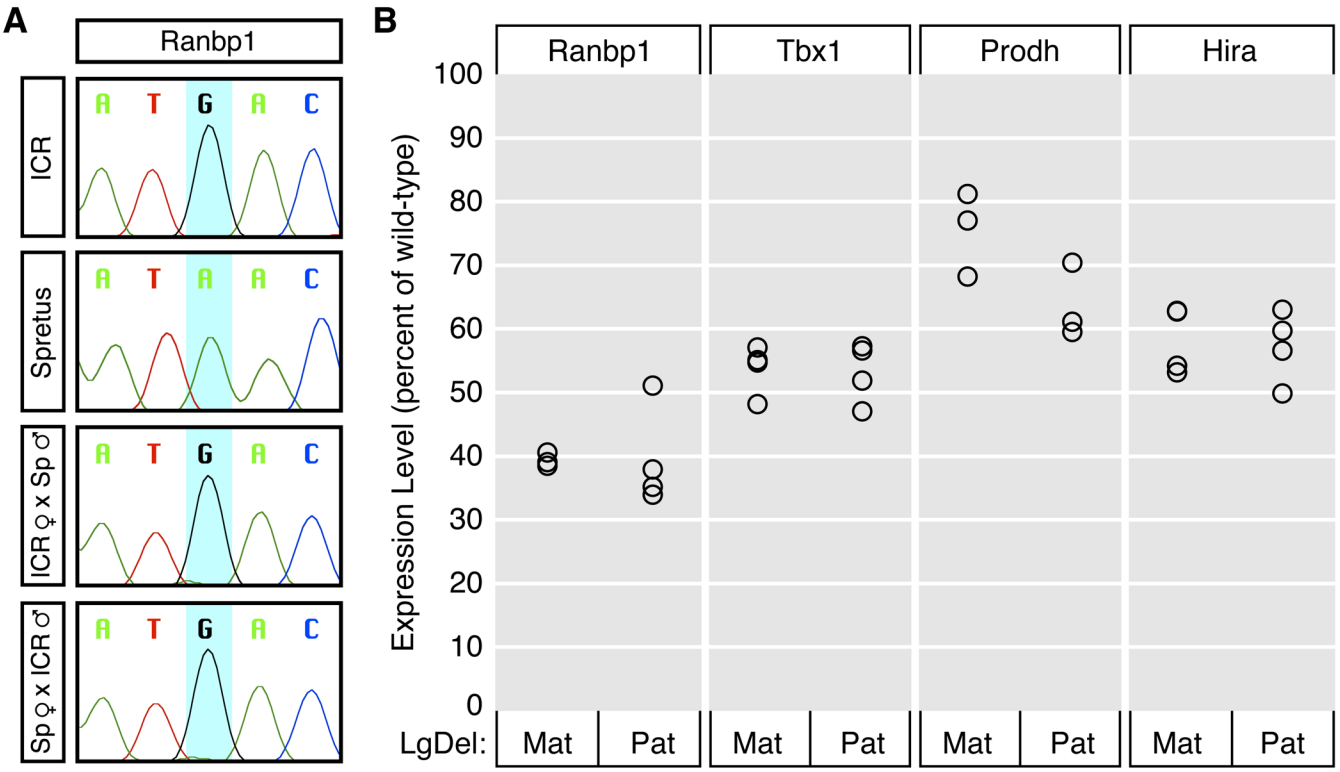

Figure 5.

qPCR measurements of 22q11 orthologues extend SNP-based analysis of allelic expression. A. Strain preference of *Ranbp1* transcription demonstrates necessity of using LgDel mouse for analysis of allelic bias for this gene. Chromatograms from sequencing of *Ranbp1* from native ICR and *spretus* show a polymorphic base; however, the ICR allele is always predominant in interspecies crosses. B. Extension (*Ranbp1*, *Tbx1*), validation (*Hira*) and lack of confirmation (*Prodh2*) of 22q11 orthologue allelic biases using quantitative RT-PCR in LgDel mice. Four maternally deleted, four paternally deleted, and eight matched control samples were measured in triplicate, relative to a GAPDH control.

PCR primers used to identify SNPs and measure expression bias for each transcript (Column 1). For reference, column 2 notes some of the alternate names used in published literature for some of these transcripts. Columns 3 and 4 list the forward and reverse oligonucleotide sequences in 5′->3′ direction. Column 5 notes which primer (forward or reverse) was used for sequencing, while column 6 notes the length of the amplified transcript. Column 7 lists the identity of the sequence polymorphisms that were measured, and their location on the sequenced strand.

Gene	Alternate Name	Forward Primer	Reverse Primer	Seq. strand	Amplicon size	Location of analyzed SNP (icr→spret)
Arvcf		gagggcccgccatcttgagc	caggccccgaggttacttgagg	f	538	229 t→g
Bcr		catggccttccgcgttcacagtc	ccggcgctcgatctcttccacac	f	751	295 c→g
Cdc45L		cagagaagcgcacacggttagaagag	cccatgtctgcaaggaactcctggag	f	496	254 c→t
Cldn5	TMVCF	ctggaccacaacatcgtgac	gtacttgaccgggaagctga	f	759	406 (+AC)
Comt		ccttcctcctgctggtgcgacacc	gcgagggcctgtactcccgaatc	r	268	80 c→g; 93 a→g; 174 gc→at; 222 a→g
Crkl		ccatccccgagcccaacaccat	gtgcgggcactccaccactgct	r	514	241 a→g
D16H22S680E	T10	cgcccgtggtagaggtgaacttgt	ataggtggcacagcggacacacac	f	451	97 t→c; 121 t→c; 133 g→a; 205 c→t
Dgcr2		catcctctcgctgctgcttttcat	ccccctggcggtgcttctgta	f	537	214 a→g; 271 a→g; 367 a→g
Dgcr6		gagactgcggctgcagaacgaacac	ggcccttcccctccaaatctgaac	r	529	340 c→t; 364 a→t
Dgcr8		gaggcccatcctgccagccaac	cgctctgcagccagcctcttcatc	r	801	153 c→t
E2f6		gagcggctagtagcagtgcagagtgac	gctttgaggcatgccaccaatcttc	f	802	259 c→t
Es2el	DGCR1	cggcacgcgtggctctacc	ggggctctcggatccttctactcg	f	474	312 c→t; 318 t→c
Gnb1l	WDVCF	cagggaagggcagcgacgaggtt	ccccgcagcaagcaagccatcag	r	539	127 t→c
Hira		ctgcaagggcaggaccacactattg	ccccggctcctgctctcacaaatg	f	367	155 a→g;298 a→t
Htf9c		ctgcgcccccactatgtcaaaaag	ccagggtagcagggcacgattagt	f	533	104 a→c
Mkrn3	Zfp127	ccccctgctgcatcctcaagctc	tggccaggcgaagcacagaatg	f	1017	159 a→g; 366 g→t; 325 + (gtgcca);
Mrpl40	NLVCF	gtgtgctgcgcgggctctg	tctcgaaggggaataggctggta	f	497	133 g→a; 145 g→a
Prodh	ProDH2	gcttctggggcaaaggatgttcgag	gtcaggcggctgatggctggttg	f	888	365 a→g;397 c→t
Ranbp1		gcggaggaaaggagctgcgac	ccactatggaaaaagaaaagttagcac	f	483	206 t→a; 271 g→a; 420 g→a
Rtn4r	NoGoR	gccgcctcatgaccctctacctg	cctctgcgtcccctgttccactg	f	660	254 t→c; 410t→c
Sept5	CDCrel1	gcaccgcaagtccgtcaag	gggcaccaggcagtcagc	f	487	181 c→t
Slc25a1	CTP	ctgcgtgcggcaaactgtcc	ggccctgcatcctggtcttg	r	545	194 c→g; 200 a→g
Txnrd2	ThioR2	ggagccctggaatatggaatcaca	ggccgcccctcagcaacatc	f	497	264 c→t
Ufd1l		caactcagccggctcaacattacc	agaaccagagaaggcacggaagc	f	531	147 g→t

Gene	Alternate Name	Forward Primer	Reverse Primer	Seq. strand	Amplicon size	Location of analyzed SNP (icr→spret)
Zdhhc8		cgggatgcttgggggtgaggattc	gcccggcacacgctggttaag	r	867	246 c→t

SNP frequencies for 25 murine 22q11 orthologues. For each transcript, the table indicates the number of polymorphisms identified (single nucleotide and insertion/deletion polymorphisms), as well as the total number of bases sequenced. Only some polymorphisms were suitable for measurement analysis, thus the number of identified polymorphisms in many transcripts is greater than shown in Table 1. The total bases sequenced and an estimate of the average frequency of polymorphisms in cDNA transcripts is noted at the end of the table.

	SNP	Insertion	bases sequenced
Arvcf	1		469
Bcr	1		687
Cdc45l	1		1423
Cldn5	0	1 (2 bp)	682
Comt	9		483
Crkl	2		450
D16H22S680E	4		368
Dgcr2	3		473
Dgcr6	3		433
Dgcr8	1		710
E2f6	3		728
Es2el	2		405
Gnb1l	1		493
Hira	2		1083
Htf9c	1		465
Mrpl40	2		431
Prodh	2		562
Ranbp1	3		583
Rtn4r	2		834
Sept5	1		428
Slc25a1	2		474
Tbx1	0		1514
Txnrd2	1		426
Ufd1l	1		481
Zdhhc8	3		797
Mkrn3	2	1 (6 bp)	938
Average	1 SNP every 317 bp	1 insertion/deletion every 8410 bp	